iDamage: a method to integrate modified DNA into the yeast genome

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Abstract

In order to explore the mechanisms employed by living cells to deal with DNA alterations, we have developed a method by which we insert a modified DNA into a specific site of the yeast genome. This is achieved by the site-specific integration of a modified plasmid at a chosen locus of the yeast genome, through the use of the Cre/lox recombination system.

In the present work, we have used our method to insert a single UV lesion into the yeast genome, and studied how the balance between error-free and error-prone lesion bypass is regulated. We show that the inhibition of homologous recombination, either directly (by the inactivation of *rad51* recombinase) or through its control by preventing the poly-ubiquitination of PCNA (*ubc13* mutant), leads to a strong increase in the use of TLS. Such regulatory aspects of the DNA damage tolerance could not have been observed with previous strategies using plasmid or randomly distributed lesions, which shows the advantage of our new method.

The very robust and precise integration of any modified DNA at any chosen locus of the yeast genome that we describe here is a powerful tool that will allow exploration of many biological processes related to replication and repair of modified DNA.

Introduction

Various exogenous and endogenous agents pose a constant threat to the genome of all organisms, resulting in DNA modifications such as abasic sites, DNA adducts [1], DNA crosslinks (intra- or inter-strand), DNA-protein crosslinks [2], presence of ribonucleotides [3], etc. Unrepaired, these modifications present a serious challenge to a cell, as they may impair replication or give rise to deleterious mutations. In response to those threats, organisms have evolved many different mechanisms to deal with DNA damage.

Numerous repair systems exist that remove various modifications from DNA in an error-free manner [4]. However, despite their efficient action, it is inevitable that some damages will persist long enough to be present during replication, which can lead to replication defects (replication blocks or delays, fork collapse, etc.) or alter replication fidelity. Therefore, to complete replication and maintain cell survival in the presence of residual DNA damages, cells have evolved two DNA Damage Tolerance (DDT) mechanisms: i) Translesion Synthesis (TLS), employing specialized DNA polymerases able to insert a nucleotide directly opposite the lesion. This pathway is potentially mutagenic due to the miscoding nature of most damaged nucleotides and to the low fidelity of the TLS polymerases (reviewed in [5]); ii) Damage Avoidance (DA, also named strand switch, copy choice or homology directed gap repair), a generally error-free pathway where the cells use the information of the sister chromatid to circumvent the lesion in an error-free manner (reviewed in [6]). The balance between error-free and error-prone mechanisms is important since it defines the level of mutagenesis during lesion bypass.

Decades of studies of DNA damage tolerance have yielded significant advances in our knowledge. It is well established that in eukaryotic cells, error-prone TLS is controlled by PCNA mono-ubiquitination while error-free DA is triggered by PCNA poly-ubiquitination. However, several questions remain regarding how the balance between these two pathways is controlled.

Over the years, many assays have been developed to study TLS and DA. Yet, their main limitation is that they do not allow to monitor both TLS and DA at the same time. Genome-wide assays involving treatment with DNA damaging agents allows to monitor toxicity and mutagenesis, but are blind to error-free events. The introduction of single lesions onto replicative plasmids have been successfully used to monitor error-free and error-prone TLS [7-12]. However, plasmid-based assays are not suited for the analysis of DA events, as during plasmid replication, when a lesion is encountered, replication fork uncoupling leads to full separation of the daughter strands in plasmids, while DA events require close proximity of the two sister chromatids [13].

To overcome the limitations of these approaches, we designed an assay to follow the fate of a single replication-blocking lesion introduced in the genome of a living cell. Our group has previously developed such assay in *Escherichia coli* [13,14], and showed that indeed, such approach allows to monitor both TLS and DA

events, and the interplay between these two tolerance mechanisms. It appeared necessary to develop a similar approach in eukaryotic cells in order to explore DNA damage tolerance in this kingdom of life. We chose to use the yeast *Saccharomyces cerevisiae* which provides an invaluable model due to the ease of genetic manipulation and high homology with several human genes. Furthermore, recent progress in construction of yeast strains with humanized genes and pathways opens up many possibilities for the study of human genes and processes in a simpler organismal context [15].

The method described here involves the site-specific integration of a vector containing a single DNA modification and a strand marker that allows to distinguish the modified from the non-modified strand upon replication. A simple colorimetric assay permits to monitor TLS and DA events.

We have used our method to insert two different UV lesions into the genome of the yeast *Saccharomyces cerevisiae*. We confirm the involvement of several specialized DNA polymerases that has previously been observed using randomly distributed lesions and plasmid assays. In addition, we show that impairing the DA pathway either at the control level (*ubc13*) or at the effector level (*rad51*), leads to an increase in the use of both error-free and mutagenic TLS. Such interplay between TLS and DA has never been observed before as it can only be evidenced on the chromosomal DNA and at the level of a single lesion. It shows the advantage of our method over currents approaches relying on plasmid-based assays or lesions randomly distributed over the genome.

Results

1. Site-specific integration into the yeast genome

In order to overcome the limitations previously described, we developed an assay that allows to follow the fate of a single replication-blocking lesion in the yeast genome. This technique is based on a non-replicative plasmid containing a single lesion, which is stably integrated into one of the chromosomes using site-specific recombination, as previously described for *E. coli* [13,14] (Fig.1). After testing several integration strategies (see supplementary information), we chose a modified version of the Cre/lox system involving Left-Element/Right-Element (LE/RE) lox site mutants (Supplementary Fig.1). Recombination between LE (lox61) and RE (lox71) lox mutants produces a wild-type loxP site as well as a LE+RE double mutant lox site that is not recognized by Cre [16], thus preventing excision of the plasmid once integrated into the chromosome. In addition, if several plasmids enter the cell, once one of them is integrated, the remaining ones cannot be exchanged on the chromosome. Additionally, we placed the Cre recombinase under the control of the doxycycline repressible promoter (Tet-off) so it can be turned off after integration has occurred.

Following ectopic expression of Cre recombinase (pKM34), the plasmid carrying a lesion is introduced by electroporation into a recipient *S. cerevisiae* strain containing a chromosomal integration cassette. The plasmid contains a selectable marker (LEU2), and a single lesion located within the 5-end of the *lacZ* gene fused to a lox71 site. The chromosomal integration site contains the 3-end of *lacZ* fused to lox66 site, so that following the precise integration a full-length functional ß-galactosidase gene (*lacZ*) is restored (Fig.1, Fig.S1).

We placed the chromosomal integration site close to an early replication origin (ARS306 or ARS606) in two different orientations in order to introduce the lesion either on the leading or the lagging strand (Supplementary Fig.S1). The non-damaged strand contains a +2 frameshift inactivating the *lacZ* gene, serving as a genetic marker to allow strand discrimination. After electroporation of the vector, cells are plated on selective indicator plates (SD-LEU, X-gal) before the first cell division occurs. The lesion is placed in such sequence context, that all in-frame TLS events, both error-free and mutagenic, result in a functional *lacZ* gene (blue colony sectors), while DA events result in inactivated *lacZ* gene (white colony sectors).

PCR analysis and sequencing confirmed that all colonies obtained on selective plates result from precise integration of the vector into the chromosomal integration site. No colonies were observed after transformation of a strain not expressing Cre recombinase or without chromosomal integration site.

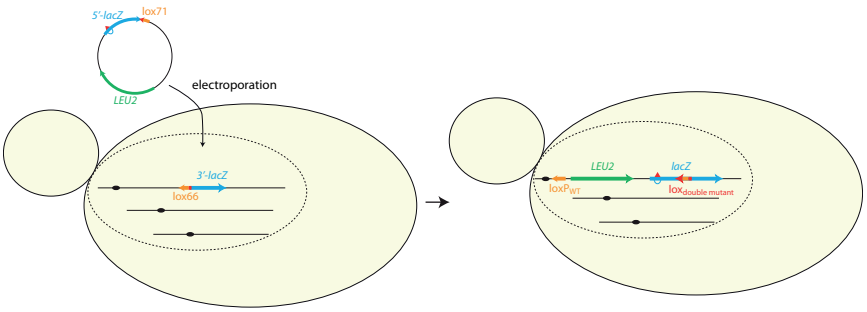

Figure 1. A non-replicative plasmid containing a single lesion is integrated into one of the yeast chromosome (III or VI) using Cre/lox site-specific recombination. The integrative vector carrying a selection marker (LEU2) and the 5'-end of the *lacZ* reporter gene containing a single lesion is introduced into a specific locus of the chromosome with the 3'-end of the *lacZ*. The precise integration of the plasmid DNA into the chromosome restores a functional *lacZ* gene, allowing the phenotypical detection of TLS and DA events (as blue and white colonies on X-gal indicator media). Plasmid pRS413 (containing HIS3 marker) is co-transformed and used as an internal control to normalize transformation efficiency between damaged and non-damaged vectors.

2. Bypass of UV lesions by Translesion synthesis

To validate our system, we constructed 3 integration vectors, containing no lesion, a TT-CPD lesion (cyclobutane pyrimidine dimer) and a TT(6-4) lesion (thymine-thymine pyrimidine(6-4)pyrimidone photoproduct). In order to focus on lesion tolerance mechanisms, we inactivated the repair mechanisms in our parental strain (namely nucleotide excision repair: *rad14*, and photolyase: *phr1*), as well as the mismatch repair system (*msh2)*, to avoid the repair of the strand marker. Tolerance events are calculated as the ratio of colonies resulting from the integration of the damaged vector versus the lesion-free one.

After integration of the constructs containing a single CPD or TT(6-4) lesion, no reduction of survival was observed compared to the lesion-free construct (Fig.2). Integration of the heteroduplex containing a single CPD lesion leads to 55% of sectored blue colonies representing TLS events. For TT(6-4) lesion, 4% of TLS events were observed. Those results are in agreement with a previous report by Gibbs et al. [9], where the authors used gapped-circular vectors containing a single lesion within a short single-stranded region. We observed no differences between the leading and lagging strand in the ratio of TLS and DA.

In the absence of Pol η (*rad30*), TLS at CPD lesion is strongly reduced to ~18%. The remaining TLS in the absence of Pol η is dependent on *REV1* and *REV3* as inactivation of either of these genes in combination with *rad30* leads to an almost complete suppression of TLS events. Despite the drop of 63% in the rate of TLS at the CPD in the absence of Pol η, we observe no loss of survival. This is consistent with the study by Abdulovic and Jinks-Robertson [17] where the authors demonstrated that *rad30* strain is not sensitive to low UV doses. We can therefore conclude that in the presence of low levels of DNA damage homologous recombination-dependent mechanism (DA) can fully compensate for the absence of specialized polymerase.

In the presence of Pol η, inactivation of either *REV1* or *REV3* leads to a milder reduction of TLS at a CPD lesion. Both genes are epistatic as the inactivation of both *rev3* and *rev1* leads to the same decrease of TLS. It is interesting to note that Pol η-mediated TLS and Rev1-Rev3-mediated TLS are independent from each other and seem compartmentalized: the drop in TLS in the absence of Pol η cannot be compensated by Rev1-Rev3 TLS and vice versa.

RAD30 inactivation has no effect on TLS at TT(6-4) lesions. However, *REV1* or *REV3* (or both) inactivation leads to a complete suppression of TLS at this lesion, showing again the epistasis of both genes in the bypass of this lesion.

Molecular analysis of colonies obtained after lesion integration (Supplementary Table S2) shows that insertion opposite the CPD lesion is 100% error-free in the presence of Pol η, as expected from the specificity of this polymerase for this lesion [18]. However, it is interesting to note that even in the presence of Pol η, the bypass of CPD is mutagenic in 18% of the cases due to mis-elongation by Rev1-Polζ. The insertion step becomes mutagenic (5%) in the absence of Pol η.

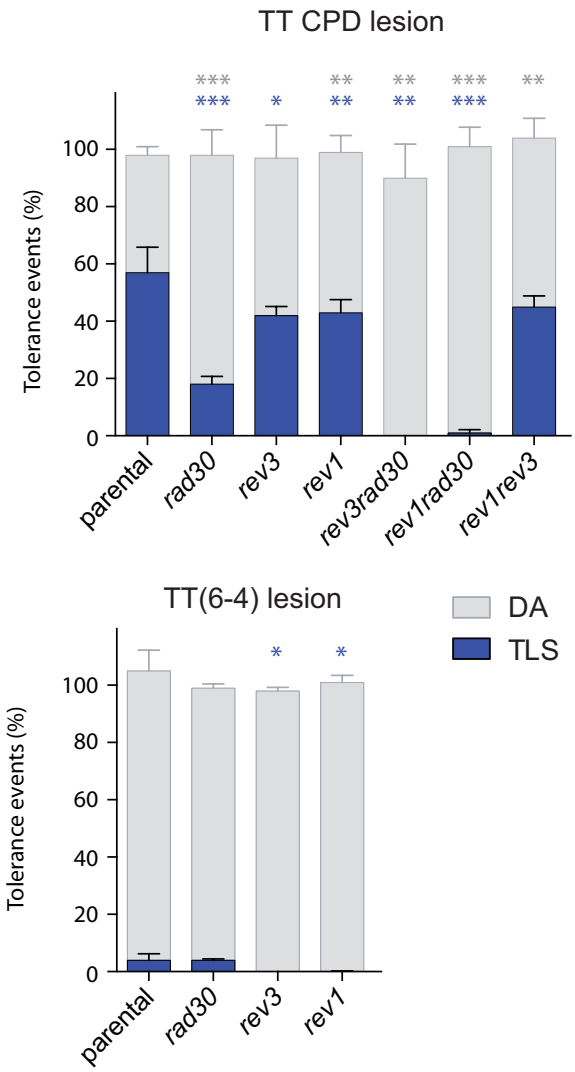

Figure. 2: Partitioning of DDT pathways in a strain deficient in TLS polymerases

The graph represents the partition of DDT pathways for two UV lesions, in strains deficient in TLS polymerases (Rev1, pol η/*rad30*, pol ζ/*rev3*). Tolerance events represent the percentage of cells able to survive in presence of the integrated lesion compared to the lesion-free control. The data represent the average and standard deviation of at least three independent experiments in which the lesion has been inserted either in the leading or the lagging strand. Unpaired *t*-test was performed to compare TLS and DA values from the different mutants to the parental strain. **P* < 0.05; ***P* < 0.005; ****P* < 0.0005.

A. In a parental strain over 50% of events observed across a CPD lesion are TLS events. DNA polymerase is responsible for the majority of CPD lesion bypass. However, in its absence TLS bypass of this lesion is still possible through the combined action of polymerase and Rev1. In the absence of pol η, removal of either Rev1 or pol ζ completely abolishes TLS.

B. For the TT(6-4) lesion, DA is the major tolerance pathway. Majority of TLS events through TT(6-4) lesion depends on pol ζ and Rev1, while pol η rarely contributes to the bypass of this lesion.

Overall mutagenesis (both at the insertion and elongation steps) is almost completely abolished in the absence of *rev3* and *rev1*. The bypass of the TT(6-4) lesion is mutagenic in 30% of the case, mostly due to misincorporation at the insertion step.

Altogether, these results confirm the involvement of TLS polymerases in the bypass of UV lesion that was previously obtained with replicative or gapped plasmids [9,12]. They show that our method by which we introduce a single lesion in the genome is suitable to study TLS. In addition, we show that a decrease in TLS caused by the absence of one (or several) specialized DNA polymerase(s) is fully compensated by a concomitant increase in the DA process, avoiding any decrease in the cell survival.

3. PCNA mono-ubiquitination is required for TLS

It is known that the balance between TLS and DA is regulated by post-translational modifications of PCNA that occur in response to DNA damage. PCNA mono-ubiquitination (at Lysine 164) is mediated by Rad6/ Rad18 and favors TLS [19]. PCNA poly-ubiquitination depends on Mms2-Ubc13 ubiquitin-conjugating complex and the ubiquitine-ligase activity of Rad5, and is important for DA [20]. Since our system was designed to explore the balance between error-prone and error-free lesion bypass pathways, we investigated the role of PCNA ubiquitination on the bypass or our UV lesions. We introduced our two UV lesions in strains were PCNA cannot be ubiquitinated, either by the inactivation of *RAD18*, or by the mutation of Lysine 164 of PCNA (*pol30*-K164R) (Fig.3). In both situations, in the absence of PCNA ubiquitination, the TLS level at CPD and TT(6-4) lesions is almost completely abolished. It appears therefore that PCNA ubiquitination is an absolute requirement for TLS.

Interestingly, in the absence of any DNA-damaging treatment, the presence of a single replication-blocking lesion is sufficient to generate the signal required to trigger Rad6-Rad18-mediated PCNA ubiquitination which is clearly necessary to promote TLS.

It is also interesting to note that DA is still possible in the absence of PCNA ubiquitination since we still observe a great proportion of white colonies in the *rad18* and the *pol30-K164R* mutant. However, while the drop in TLS caused by the absence of one or multiple DNA polymerases was fully compensated by DA mechanisms (Fig. 2 and previous paragraph), the drop of TLS induced by the lack of PCNA ubiquitination can only be partially compensated by DA, leading to a reduced survival (Fig. 3). This shows that DA partially depends on PCNA ubiquitination.

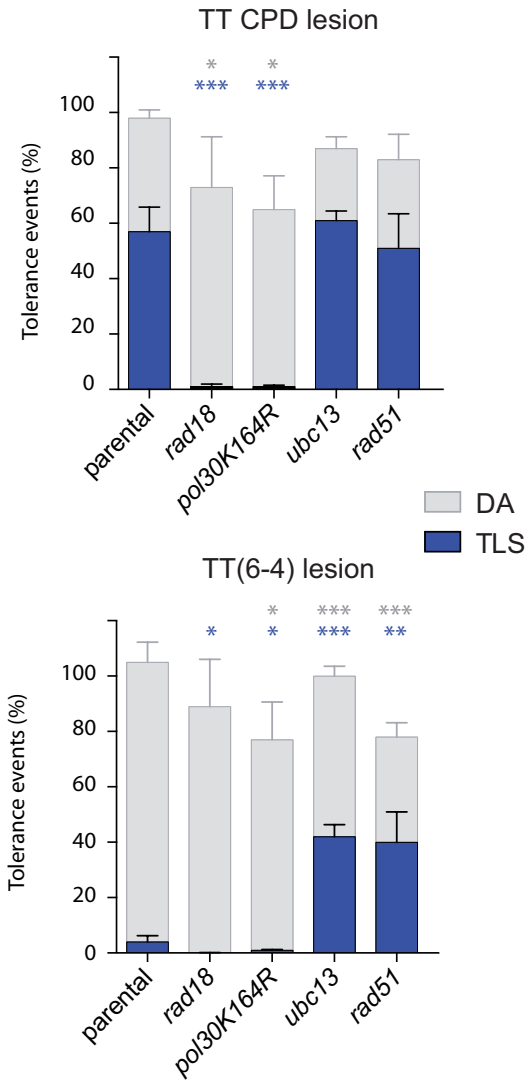

Figure. 3: Partitioning of DDT pathways in the absence of PCNA ubiquitylation

Bypass of TT-CPD (A) and TT(6–4) (B) lesion in strains deficient in PCNA ubiquitylation (*rad18* or *pol30-K164R*) or polyubiquitylation (*ubc13*). Tolerance events represent the percentage of cells able to survive in presence of the integrated lesion compared to the lesion-free control. The data represent the average and standard deviation of at least three independent experiments in which the lesion has been inserted either in the leading or the lagging strand. Unpaired *t*-test was performed to compare TLS and DA values from the different mutants to the parental strain. $*P < 0.05$; $**P < 0.005$; $***P < 0.0005$.

In the absence of PCNA ubiquitylation (*rad18, pol30 K164R*) we observed a decrease in TLS. The remaining low level of TLS is probably due to polymerase interactions with PCNA ring not involving ubiquitin moiety. In the absence of PCNA polyubiquitylation (*ubc13*), a small increase of the TLS bypass of the CPD lesion is observed, while Pol ζ-mediated bypass of the TT(6-4) lesion increased more than 10 fold. The absence of recombinase Rad51 results in a similar increase in TLS.

4. Lack of PCNA poly-ubiquitination favors TLS

We then looked at how PCNA poly-ubiquitination could affect the ratio DA/TLS (Fig.3). In the absence of PCNA poly-ubiquitination (*ubc13* strain), we observed no significant effect on the bypass of CPD lesion. On the other hand, we observed a more than 10 fold increase in the Pol ζ-mediated TLS at the TT(6-4) lesion, reaching more than 40% (Fig. 3). In the absence of PCNA poly-ubiquitination, DA is reduced and is compensated by an

increase in TLS. Such phenomenon has previously been observed in *E. coli* where we showed that a defect in homologous recombination led to increased TLS [21] using a similar approach. Only the monitoring of a single DNA lesion inserted in the genomic DNA is able to reveal such interplay between DA and TLS.

To confirm that the increase in TLS was indeed due to a decrease in DA, we inactivated the Rad51 recombinase which is a key actor in the DA mechanism [22,23]. In the *rad51* strain, we observed the same 10-fold increase in TLS rate at the TT(6-4) lesion (Fig. 3). This confirms that affecting the DA process, either at its regulation level (*ubc13*) or at its effector level (*rad51*) leads to an increase in TLS.

As we did not observe a similar increase in TLS at the CPD lesion, we hypothesized that the competition between DA and TLS occurs behind the fork, during a gap filling reaction. Following the encounter with a blocking lesion, a repriming event generates a single-strand DNA gap that will be filled post-replicatively [24,25]. The majority of CPD lesions is efficiently bypassed by Pol η at the fork. Only the small fraction that is bypassed by Pol ζ behind the fork, is in competition with *UBC13*-dependant DA mechanisms for the gap-filling reaction. This hypothesis will need to be confirmed by the use of other strongly-blocking lesions that are bypassed by Pol ζ. In the *ubc13* or *rad51* strains, some DA still persists as we still observe a significant number of white colonies. By adding markers on the damaged and non-damaged strand, we will be able to explore and characterize these DA events as previously achieved in *E. coli* [26].

Discussion / Conclusion

Our goal was to develop a method to explore the mechanisms employed by living cells to deal with DNA alterations. Over the years, many assays have been developed to study error-prone TLS or error-free DA. However, their main limitation is that they did not allow to monitor both TLS and DA simultaneously. Assays measuring chromosomal mutagenesis after treatment with mutagenic agents are blind to error-free events. Plasmid-based systems have been successfully used to monitor error-free and error-prone TLS [7-12]. They are, however, not suited for the analysis of DA events [13].

In the present paper, we describe a method that overcomes these limitations by allowing to monitor the fate of a single DNA modification inserted in the genome of a yeast cell. We have used this method to introduce a single UV lesion (TT(6-4) or CPD) into the genome of *S. cerevisiae* and studied its bypass by DNA damage tolerance pathways. Several factors have been proposed to regulate the interplay between TLS and DA, among them the nature of the lesion and the post-translational modification of PCNA. However, no high-resolution assays were able to monitor both TLS and DA simultaneously at the level of a single lesion in the genome. Using our method, we were able to show that the proportion of TLS *vs.* DA is dependent on the lesion: while TLS represents ~55% of the tolerance pathways for CPD, it represents only ~4% for TT(6-4). For both lesions, no toxicity is observed and DA complements TLS pathway in order to recover 100% of survival (as compared to the integration of the lesion-free control vector). We showed that in the absence of TLS polymerases, DA mechanisms could fully compensate for the lack of TLS avoiding any drop in survival. However, when PCNA ubiquitination was abolished, TLS was almost completely suppressed but could only be partially compensated by DA, showing that DA partially depends on PCNA ubiquitination.

More interestingly, we showed that a defect in the DA pathway leads to an increase in TLS. Indeed, at the TT(6-4) lesion, when the DA pathway is affected either by the inactivation of *ubc13* or of *rad51*, it is compensated by a 10 fold increase in TLS. This increase of TLS due to a defect in DA can only be revealed by our method. Previously used approaches based on randomly distributed lesion (e.g. UV irradiation) could reveal an increase in mutagenesis, but were blind to error-free processes (including DA and error-free TLS). It has previously been reported that *ubc13* inactivation led to a ~2-fold increase in UV-induced mutagenesis [27], reflecting the low fraction of mutagenic TLS events. We report here a >10-fold increase in the use of Pol ζ-mediated TLS in the *ubc13* strain, our system allowing to monitor both mutagenic and error-free TLS events. Plasmid-based assays that allow to monitor error-free TLS have been used, but they are inappropriate substrates to monitor DA: due to their limited size, the full unwinding of the two DNA strands prevents homologous recombination with the sister chromatid as previously evidenced in *E. coli* [13]. Indeed, the inactivation of *ubc13* doesn't lead to any increase of TLS at a single lesion bypassed on a plasmid system in *S. cerevisiae* [28].

The increase of TLS in response to inhibition of DA evidenced here could not be observed before in yeast since no assay was able to simultaneously monitor TLS and DA, and therefore the interplay between these two mechanisms. The method described here has the potential to unveil several new aspects of the DNA damage tolerance. Many factors may affect the DNA damage tolerance pathway choice, including lesion type, sequence context, location in the genome, chromatin state, cell cycle stage, and transcriptional activity. The versatility of our assay permits to explore the impact of those factors. Our assay allows to insert any type of DNA lesion or modification at any desired location in the yeast genome. By placing the damage in centromeric or telomeric regions, highly/poorly transcribed regions, hetero/eu-chromatin regions, near

fragile sites etc…, it will be possible to determine how these positions affect the balance between error-free and mutagenic lesion bypass. In addition, the integration site can be placed in two orientations relative to the closest replication origin, allowing to place the lesion on the leading or lagging strand. Similarly, we can choose to insert the lesion on the transcribed, or on the non-transcribed strand.

This method opens the way to exploration of lesion bypass and single-strand gap repair in the same manner engineering nucleases such as HO or I-SceI has allowed to boost the exploration of double strand breaks repair [29].

This method is not limited to DNA Damage tolerance, but can also be used to explore several mechanisms related to DNA maintenance, such as repair of DNA adducts, repair of DNA crosslinks, effect of ribonucleotides inserted into the DNA [3], effect of DNA-protein crosslinks [2], etc… The possibility to integrate vectors carrying any type of damage or chemical group broadens the applicability of our approach beyond the field of DNA damage repair and tolerance. Being able to locate a single modification at a specific locus of the genome will enable powerful molecular analysis at the resolution of a single replication fork.

References

1. Friedberg, E. C. *DNA Repair And Mutagenesis*. (Amer Society for Microbiology, 2006).
2. Stingele, J., Bellelli, R. & Boulton, S. J. Mechanisms of DNA-protein crosslink repair. *Nat Rev Mol Cell Biol* **18,** 563–573 (2017).
3. Lazzaro, F. *et al.* RNase H and Postreplication Repair Protect Cells from Ribonucleotides Incorporated in DNA. *Molecular Cell* **45,** 99–110 (2012).
4. Friedberg, E. C. Suffering in silence: the tolerance of DNA damage. *Nat Rev Mol Cell Biol* **6,** 943–953 (2005).
5. Waters, L. S. *et al.* Eukaryotic translesion polymerases and their roles and regulation in DNA damage tolerance. *Microbiol Mol Biol Rev* **73,** 134–154 (2009).
6. Branzei, D. & Szakal, B. DNA damage tolerance by recombination: Molecular pathways and DNA structures. *DNA Repair (Amst)* (2016). doi:10.1016/j.dnarep.2016.05.008
7. Pagès, V. & Fuchs, R. P. Uncoupling of leading- and lagging-strand DNA replication during lesion bypass in vivo. *Science* **300,** 1300–1303 (2003).
8. Yoon, J.-H., Prakash, L. & Prakash, S. Highly error-free role of DNA polymerase eta in the replicative bypass of UV-induced pyrimidine dimers in mouse and human cells. *Proc Natl Acad Sci USA* **106,** 18219–18224 (2009).
9. Gibbs, P. E. M., McDonald, J., Woodgate, R. & Lawrence, C. W. The relative roles in vivo of Saccharomyces cerevisiae Pol eta, Pol zeta, Rev1 protein and Pol32 in the bypass and mutation induction of an abasic site, T-T (6-4) photoadduct and T-T cis-syn cyclobutane dimer. *Genetics* **169,** 575–582 (2005).
10. Gibbs, P. E. & Lawrence, C. W. Novel mutagenic properties of abasic sites in Saccharomyces cerevisiae. *J Mol Biol* **251,** 229–236 (1995).
11. Bresson, A. & Fuchs, R. P. P. Lesion bypass in yeast cells: Pol eta participates in a multi-DNA polymerase process. *Embo J* **21,** 3881–3887 (2002).
12. Pagès, V., Johnson, R. E., Prakash, L. & Prakash, S. Mutational specificity and genetic control of replicative bypass of an abasic site in yeast. *Proc Natl Acad Sci USA* **105,** 1170–1175 (2008).
13. Pagès, V., Mazon, G., Naiman, K., Philippin, G. & Fuchs, R. P. Monitoring bypass of single replication-blocking lesions by damage avoidance in the Escherichia coli chromosome. *Nucleic Acids Res* **40,** 9036–9043 (2012).
14. Pagès, V. & Fuchs, R. P. Inserting Site-Specific DNA Lesions into Whole Genomes. *Methods Mol Biol* **1672,** 107–118 (2018).
15. Laurent, J. M., Young, J. H., Kachroo, A. H. & Marcotte, E. M. Efforts to make and apply humanized yeast. *Brief Funct Genomics* **15,** 155–163 (2016).
16. Araki, K., Okada, Y., Araki, M. & Yamamura, K.-I. Comparative analysis of right element mutant lox sites on recombination efficiency in embryonic stem cells. *BMC Biotechnol.* **10,** 29 (2010).
17. Abdulovic, A. L. & Jinks-Robertson, S. The in vivo characterization of translesion synthesis across UV-induced lesions in Saccharomyces cerevisiae: insights into Pol zeta- and Pol eta-dependent frameshift mutagenesis. *Genetics* **172,** 1487–1498 (2006).
18. Johnson, R. E., Prakash, S. & Prakash, L. Efficient bypass of a thymine-thymine dimer by yeast DNA polymerase, Poleta. *Science* **283,** 1001–1004 (1999).
19. Bailly, V., Lauder, S., Prakash, S. & Prakash, L. Yeast DNA repair proteins Rad6 and Rad18 form a heterodimer that has ubiquitin conjugating, DNA binding, and ATP hydrolytic activities. *J Biol Chem* **272,** 23360–23365 (1997).
20. Stelter, P. & Ulrich, H. D. Control of spontaneous and damage-induced mutagenesis by SUMO and ubiquitin conjugation. *Nature* **425,** 188–191 (2003).
21. Naiman, K., Pagès, V. & Fuchs, R. P. A defect in homologous recombination leads to increased translesion synthesis in E. coli. *Nucleic Acids Res* **44,** 7691–7699 (2016).
22. Sung, P., Krejci, L., Van Komen, S. & Sehorn, M. G. Rad51 recombinase and recombination mediators. *J Biol Chem* **278,** 42729–42732 (2003).

23. Sung, P., Trujillo, K. M. & Van Komen, S. Recombination factors of Saccharomyces cerevisiae. *Mutat Res* **451,** 257–275 (2000).
24. Lopes, M., Foiani, M. & Sogo, J. M. Multiple mechanisms control chromosome integrity after replication fork uncoupling and restart at irreparable UV lesions. *Mol Cell* **21,** 15–27 (2006).
25. Novarina, D., Amara, F., Lazzaro, F., Plevani, P. & Muzi-Falconi, M. Mind the gap: keeping UV lesions in check. *DNA Repair (Amst)* **10,** 751–759 (2011).
26. Laureti, L., Demol, J., Fuchs, R. P. & Pagès, V. Bacterial Proliferation: Keep Dividing and Don't Mind the Gap. *PLoS Genet* **11,** e1005757 (2015).
27. Halas, A. *et al.* The roles of PCNA SUMOylation, Mms2-Ubc13 and Rad5 in translesion DNA synthesis in Saccharomyces cerevisiae. *Mol Microbiol* **80,** 786–797 (2011).
28. Pagès, V. *et al.* Requirement of Rad5 for DNA polymerase zeta-dependent translesion synthesis in Saccharomyces cerevisiae. *Genetics* **180,** 73–82 (2008).
29. Sugawara, N. & Haber, J. E. Monitoring DNA recombination initiated by HO endonuclease. *Methods Mol Biol* **920,** 349–370 (2012).